THE ABSENCE OF TRANSLATIONAL BARRIER BETWEEN *CAULOBACTER CRESCENTUS* AND *ESCHERICHIA COLI*

Kanji FUJIKI, Akio FUKUDA* and Yoshimi OKADA
Department of Biophysics and Biochemistry, Faculty of Sciences, University of Tokyo, Hongo, Tokyo 113, Japan

Received 24 April 1978

1. Introduction

Among different species of bacteria, translation of a given messenger ribonucleic acid (mRNA) varies considerably in the selection and efficiency of initiation at different cistrons [1]. Two clear cases of this species difference in translational ability have been reported [2,3]. While the *Escherichia coli* 30 S ribosomal subunit initiates translation of the coat cistron of RNA phage f2 with a high efficiency, the *Bacillus stearothermophilus* 30 S subunit initiates translation primarily at the cistron of f2 A protein [2]. The RNA of the *Caulobacter crescentus* RNA phage ϕCb5 can be translated by the *C. crescentus* 30 S subunit but not at all by the *E. coli* 30 S subunit, whereas the converse is true for the RNA of the *E. coli* RNA phage MS2 [3]. The source of the 50 S subunit and initiation factors have no effect on these species differences. In the latter case, it is implied that there exists a rather exclusive translational barrier between the two gram-negative bacteria.

We reconsider here the species barrier of translation between *E. coli* and *C. crescentus*. Contrary to the previous observation with the RNA from the *C. crescentus* RNA phage ϕCb5 [3], the RNA from a different *C. crescentus* RNA phage, ϕCp2, was translated with high efficiency in an *E. coli* (Q13) cell-free protein-synthesizing system. From the analysis of the major in vitro products, it appeared that phage ϕCp2 RNA can be translated correctly in the heterologous *E. coli* system.

* To whom correspondence should be addressed

2. Materials and methods

2.1. *Bacterial and phage strains, and growth conditions*

C. crescentus CB13B1a, *C. crescentus* RNA phage ϕCp2, *E. coli* Q13 and *E. coli* RNA phage MS2 were used. These strains were grown and propagated as in [4,5]. The properties of phage ϕCp2 were described in [4].

2.2. *In vitro protein synthesis*

Cell-free protein-synthesizing S30 extracts were prepared from *E. coli* cells as in [6]. The reaction mixture (0.2 ml) for protein synthesis at 30°C contained 80 mM Tris–HCl buffer (pH 7.8); 8 mM magnesium acetate; 50 mM KCl; 6 mM β-mercaptoethanol; 1 mM ATP; 0.1 mM GTP; 5 mM phosphoenolpyruvate; 1 mM each of Asn, Cys, Gln, Met, and Trp; 0.1 mM each of Ala, Arg, Asp, Glu, Gly, Ile, Leu, His, Lys, Phe, Pro, Ser, Thr, Tyr and Val; 10 μg/ml pyruvate kinase; 2 μCi/ml of a 15 [^{3}H]amino acid mixture, algal type (New England Nuclear, Boston); 60 μg/ml phage RNA; 3 mg/ml protein of pre-incubated S30 extracts.

2.3. *Electrophoresis of proteins synthesized in vitro*

After the reaction for protein synthesis, the mixture (0.2 ml) was treated with 10 μg/ml each of RNases T1 and T2 for 30 min at 37°C, adjusted to 0.5 M urea, 0.05 M β-mercaptoethanol, 1% sodium dodecyl sulfate (SDS) and 0.05 M ethylenediaminetetraacetate, and incubated for 60 min at 37°C. The mixture was then passed through a 0.9 × 40 cm

column of Sephadex G-25 (Pharmacia, Uppsala) equilibrated with 20% sucrose–1% SDS–0.01 M β-mercaptoethanol. The protein fraction was precipitated with 10% trichloroacetic acid and washed 3 times with acetone. SDS–polyacrylamide gel electrophoresis was carried out as in [7]. Protein bands were traced by the Gilford linear transport system and gel slices of 2 mm thickness were incubated in 0.5 ml Soluene-350 (Packard Instrument Co., Downers Grove) for 18 h at room temperature before radioactivity counting in toluene-base scintillation fluid.

2.4. *Miscellaneous*

The procedures for the extraction and purification of phage RNAs were described in [8]. After phenol extraction and ethanol precipitation, phage RNAs were further purified by benzoylated naphthoylated DEAE-cellulose (Sigma, St Louis). The anti-φCp2 serum was described in [4]; its K-value (first-order reaction constant for φCp2) was 470. After RNases T1 and T2 treatment, the above reaction mixture for protein synthesis was passed through a 0.6 × 40 cm column of Sephadex G25 equilibrated with polypeptone yeast extract (PYE)–5 mM Tris–HCl, pH 6.8. The protein fraction, 0.1 ml, and 0.1 ml anti-φCp2 serum diluted 256-fold with PYE broth were mixed and incubated at 30°C for 15 min. The immuno-complexes formed were passed through a 0.6 × 120 cm column of Sephadex G-50 (Pharmacia, Uppsala) equilibrated with PYE–5 mM Tris–HCl, pH 6.8.

3. Results

3.1. C. crescentus *phage φCp2 RNA directs protein synthesis in an* E. coli *system*

Both phages φCp2 and φCb5 infect *C. crescentus* CB13 and are only slightly different each other in their properties [4,9,10]. From the observation with φCb5 RNA [3], it was initially expected that φCp2 RNA would also be inert in an *E. coli* cell-free protein-synthesizing system. However, φCp2 RNA efficiently directed the incorporation of [^{3}H]amino acids into acid-insoluble materials in the *E. coli* S30 extracts. The radioactivities incorporated in 15 min at 30°C were 1830 cpm/μg RNA with φCp2, and 3160 cpm/μg RNA with *E. coli* MS2.

3.2. *Analysis of in vitro products directed by φCp2 RNA*

^{3}H-labeled products synthesized in vitro by φCp2 RNA in the *E. coli* S30 extracts were separated by SDS–polyacrylamide gel electrophoresis. The majority of the ^{3}H counts co-electrophoresed with marker φCp2 coat protein (fig.1). The rest of ^{3}H counts were separated mainly into two minor peaks. The nature of these minor peaks is not clear but, in analogy to *E. coli* phage MS2, they most likely represent the products of other genes (A protein and replicase) of the phage φCp2. Likewise, the ^{3}H-labeled products synthesized in vitro by MS2 RNA in the *E. coli* S30 extracts were separated into 3 peaks which corresponded, in molecular weight, to the MS2 coat protein, A protein and replicase (data not shown).

The question now arises of whether or not φCp2 RNA is translated, in the heterologous *E. coli* S30 extracts, from legitimate initiation sites into complete φCp2 proteins. As shown above, by their electrophoretic mobility, the in vitro products seemed complete. The ^{3}H-labeled in vitro-synthesized φCp2 products were incubated with anti-φCp2 serum for

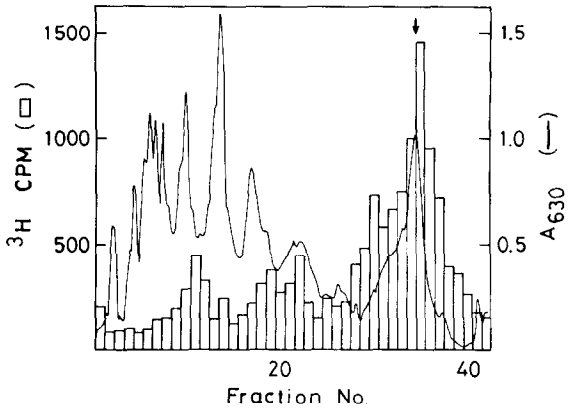

Fig.1. SDS–polyacrylamide gel electrophoresis of in vitro-synthesized φCp2 products. Protein synthesis directed by φCp2 RNA in the *E. coli* S30 extracts, SDS–polyacrylamide gel electrophoresis, and radioactivity counting were carried out as described in section 2. Marker φCp2 coat protein was added to gel samples before the run. The gel was stained with Coomassie brilliant blue and after destaining traced at A_{630} nm. The arrow indicates the position of φCp2 coat protein. The protein peaks except that of φCp2 coat protein were of ribosomal origin. Solid line, A_{630}; histogram, ^{3}H radioactivity (cpm).

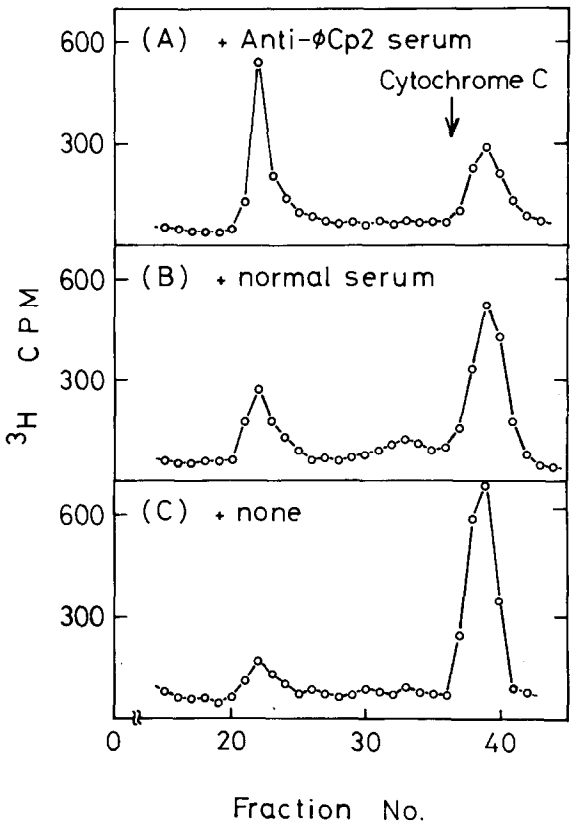

Fig.2. Sephadex G-50 column chromatography of immuno-complexes between in vitro φCp2 products and anti-φCp2 serum. The experimental procedures are described in section 2. The immuno-complexes eluted in the flow-through fractions. (A) With anti-φCp2 serum; (B) with normal serum; (C) no serum addition.

15 min at 30°C and the reaction mixture was passed through Sephadex G-50. Immuno-complexes formed with the in vitro-synthesized products should appear in the flow-through fraction. When the same total radioactivity was loaded onto each column, the ^{3}H-counts in the flow-through fraction were 63% in the presence of anti-φCp2 serum, 27% in the presence of normal serum and 18% without serum addition (fig.2). From this immunological result, and from the electrophoretic mobility of the in vitro products, the RNA from *C. crescentus* phage φCp2 appeared to be translated into complete φCp2 proteins in the heterologous *E. coli* S30 extracts.

4. Discussion

We have demonstrated in this paper that the RNA from *C. crescentus* phage φCp2 can be translated efficiently in an *E. coli* cell-free protein-synthesizing system. From the molecular weight (electrophoretic mobility in SDS-polyacrylamide) and immunological reaction with anti-φCp2 serum (fig.1,2), the in vitro products appeared to reflect correct translation of φCp2 genome in the heterologous system. This result indicates that *E. coli* ribosomes can bind *C. crescentus* phage φCp2 RNA at correct initiation sites, and that, unlike the previous contention [3], there exists no exclusive translational barrier (or 'species specificity') between these two bacteria.

The discrepancy between the present and previous results may be explained by at least two possibilities:

1. The structures (primary and secondary) of the RNAs from the *C. crescentus* phages φCp2 and previously used φCb5 are so different that φCp2 RNA can but φCb5 RNA cannot bind to *E. coli* ribosomes [11].
2. It was absolutely necessary to purify φCp2 RNA by benzoylated naphthoylated DEAE-cellulose [8]; otherwise φCp2 RNA was inert in the *E. coli* system. The phage φCb5 RNA previously used possibly contained inhibitory factors of translation in the *E. coli* system.

The current evidence indicates that the translational control of protein synthesis is exerted mainly during the initiation process. The 30 S ribosomal subunit first selects the part of the mRNA where translation should begin. The species difference of cistron selection between *E. coli* and *B. stearothermophilus* is thus attributed to 16 S RNA as well as to the protein S12 of the 30 S subunit [12]. According to [13], the 3′-end sequence of 16 S rRNA forms complementary base pairs with initiating regions on mRNA, and such complementarity is the determinant of cistron selection by the ribosomes from different bacteria. The 3′-end sequence of 16 S rRNA from *C. crescentus* ribosomes is similar to that from *B. stearothermophilus* [13]. If the notion [13] is correct, then the efficiency of initiation (if not selectivity) at cistrons of φCp2 RNA may still differ in the heterologous *E. coli* system, particularly since translation of φCp2 RNA was 58% that of MS2 RNA.

References

[1] Lodish, H. F. (1976) Ann. Rev. Biochem. 45, 39–72.
[2] Lodish, H. F. (1970) Nature 226, 705–707.
[3] Leffler, S. and Szer, W. (1973) Proc. Natl. Acad. Sci. USA 70, 2364–2368.
[4] Miyakawa, K., Fukuda, A., Okada, Y., Furuse, K. and Watanabe, I. (1976) Virology 73, 461–467.
[5] Overly, L. R., Barlow, G. H., Doi, R. H., Jacob, M. and Spiegelman, S. (1966) J. Bacteriol. 91, 442–448.
[6] Nathans, D. (1965) J. Mol. Biol. 13, 521–531.
[7] Laemmli, U. K. (1970) Nature 227, 680–685.
[8] Fujiki, K., Fukuda, A. and Okada, Y. (1978) J. Biochem. (Tokyo) in press.
[9] Bendis, I. and Shapiro, L. (1970) J. Virol. 6, 847–854.
[10] Leffler, S., Hierowski, M., Poindexter, J. S. and Szer, W. (1971) FEBS Lett. 19, 112–114.
[11] Leffler, S. and Szer, W. (1974) J. Biol. Chem. 249, 1458–1464.
[12] Held, W. A., Gette, W. R. and Nomura, M. (1974) Biochemistry 13, 2115–2122.
[13] Shine, J. and Dalgarno, L. (1975) Nature 254, 34–38.